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(54) **Stabilized enzyme compositions**

Stabilisierte Enzymzusammensetzungen

Compositions d'enzymes stabilisées

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- **FUJISHIMA M ET AL: "CONTROLLED TRIAL OF COMBINED UROKINASE AND DEXTRAN SULFATE THERAPY IN PATIENTS WITH ACUTE CEREBRAL INFARCTIO", ANGIOLOGY, WESTMINSTER PUBLICATIONS, INC, GLEN HEAD, NY, US, vol. 37, no. 7, 1 July 1986 (1986-07-01), pages 487-498, XP008170151, ISSN: 0003-3197, DOI: 10.1177/000331978603700702**

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EP 2 223 708 B1

Description

FIELD OF THE INVENTION

[0001] The present invention generally relates to enzyme compositions. More particularly, the present invention pertains to stabilized enzyme compositions, medical devices having stabilized enzyme compositions, and method of production thereof.

BACKGROUND OF THE INVENTION

[0002] Implantable medical devices such as tunneled catheters play a major role in general medicine. Within days of insertion, almost all central venous catheters are coated with a fibrin sheath, and within 30 days, most catheter-related thrombi arise. (See C Harter, HJ Salwender, A Bach, G Egerer, H Goldschmidt and AD Ho (2002) Catheter-related infection and thrombosis of the internal jugular vein in hematologic-oncologic patients undergoing chemotherapy: a prospective comparison of silver-coated and uncoated catheters. Cancer 94: 245-251.) Aside from reducing the function of the catheter, these catheter-related thrombi can cause postphlebitic syndrome in 15%-30% cases and pulmonary embolism in 11% of the cases. (See DJ Kuter (2004) Thrombotic Complications of Central Venous Catheters in Cancer Patients. The Oncologist 9: 207-216.)

[0003] To minimize the risk of thrombosis, immobilization of fibrinolytic enzymes such as Urokinase (uPA) on the surface of implantable medical devices such as tunneled catheters has been described. Examples of immobilized fibrinolytic enzymes are disclosed in U.S. Patent Nos. 4,305,926, 4,273,873, 4,378,435, and 5,380,299. Unfortunately, the fibrinolytic activity from such devices, e.g. urokinase coated hemodialysis catheter from Unitika (Blood access UK-catheter, Unitika), is retained for less than two weeks. This duration is insufficient for long term or chronic devices. A variety of agents have been conventionally utilized to stabilize fibrinolytic enzymes in solution. EP1 911 470 A1 discloses a catheter (a medical device) comprising a coating comprising immobilized: silver (antimicrobial), urokinase, and heparin. Examples of agents utilized to stabilize fibrinolytic enzymes in solution is disclosed in the following foreign patents: Japanese Patent No. JP61238732A2; European Patent No. EP0200966B1; Canadian Patent No. CA2579458AA; European Patent No. EP0391400 A2; and Japanese Patent No. JP55034082A2. However, to date, these agents have not been successfully utilized in an implantable medical device.

[0004] Accordingly, it is desirable to provide an implantable medical device having extended thrombolytic properties that is capable of overcoming the disadvantages described herein at least to some extent.

SUMMARY OF THE INVENTION

[0005] The foregoing needs are met, to a great extent, by the present invention, which discloses a medical device comprising:a base material including an immobilized fibrinolytic enzyme covalently bound to the base material and dextran sulfate, the dextran sulfate having a molecular weight that is 8 kilo dalton (kDa) to stabilize the fibrinolytic enzyme when in contact with blood of the patient, wherein the fibrinolytic enzyme includes a urokinase-type plasminogen activator (uPA).

[0006] The medical device according to the present invention may include a polyurethane base material, fibrinolytic enzyme, low molecular weight dextran sulfate, and chlorhexidine base and/or a pharmaceutically acceptable salt thereof. The amount of the fibrinolytic enzyme is sufficient to reduce the formation of blood clots in a patient. The fibrinolytic enzyme is immobilized in the base material. The low molecular weight dextran sulfate stabilizes the fibrinolytic enzyme. The chlorhexidine base and/or a pharmaceutically acceptable salt thereof is disposed in the polyurethane base material in an amount sufficient to reduce microbial growth.

[0007] Yet another embodiment of the present invention pertains to a medical device including a polyurethane base material, urokinase-type plasminogen activator (uPA), dextran sulfate having a molecular weight of 8kDa, and chlorhexidine base and/or a pharmaceutically acceptable salt thereof. The amount of the uPA is sufficient to reduce the formation of blood clots in a patient. The uPA is covalently immobilized in the base material. The low molecular weight dextran sulfate stabilizes the fibrinolytic enzyme. The chlorhexidine base and/or a pharmaceutically acceptable salt thereof is disposed in the polyurethane base material in an amount sufficient to reduce microbial growth.

[0008] Yet another embodiment of the present invention related to a method of fabricating a medical device according to any one of the claims 1-10 including:forming the medical device from at least the base material; immobilizing the fibrinolytic enzyme by covalently binding the fibrinolytic enzyme on the base material; and stabilizing the fibrinolytic enzyme with dextran sulfate having a molecular weight of 8kilo dalton (kDa), wherein the fibrinolytic enzyme includes a urokinase-type plasminogen activator (uPA).

[0009] There has thus been outlined, rather broadly, certain embodiments of the invention in order that the detailed description thereof herein may be better understood, and in order that the present contribution to the art may be better appreciated. There are, of course, additional embodiments of the invention that will be described below and which will form the subject matter of the claims appended hereto.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010]

FIG. 1 is a graph showing fibrinolytic activity of an urokinase-type plasminogen activator (uPA) immobilized on polyurethane catheter segments following incubation times ranging from 0 to 28 days.

FIG. 2 is a graph showing urokinase activity of explanted catheter segments following incubation times of 0 and 14 days.

FIG. 3 is a graph showing fibrinolytic activity of uPA immobilized on polyurethane catheter segments and stabilized with low molecular weight dextran sulfate, human serum albumin, and the amino acid arginine following incubation times ranging from 0 to 28 days.

FIG. 4 is a graph showing fibrinolytic activity of uPA immobilized on chlorhexidine coated polyurethane catheter segments and stabilized with low molecular weight dextran sulfate, human serum albumin, and the amino acid arginine following incubation times ranging from 0 to 28 days.

FIG. 5 is a graph showing fibrinolytic activity of uPA immobilized on chlorhexidine coated Tecothane® catheter segments and stabilized with high molecular weight dextran sulfate and low molecular weight dextran sulfate following incubation times ranging from 0 to 9 days.

FIG. 6 is a graph showing fibrinolytic activity of uPA immobilized on chlorhexidine coated polyurethane catheter segments and stabilized with low molecular weight dextran sulfate, human serum albumin, and the amino acid arginine following incubation times ranging from 0 to 28 days.

DETAILED DESCRIPTION

[0011] Embodiments of the invention provide an implantable medical device having extended thrombolytic properties. In various embodiments, these extended thrombolytic properties are achieved via the immobilization of Urokinase (uPA) on the surface of the implantable medical devices in the presence of dextran sulfate. Two molecular weight ranges of dextran sulfate are worth distinguishing. The first molecular weight range is generally referred to as high molecular weight dextran sulfate ("HMW-DexS") which has a molecular weight of greater than 40,000 dalton (Da) to about 500,000 Da or more. HMW-DexS has previously reported to inhibit inflammatory cell invasion around an implant. The second molecular weight range is generally referred to as low molecular weight dextran sulfate ("LMW-DexS") which has a molecular weight of less that about 40,000 Da to about 500 Da. The beneficial use of LMW-DexS having a molecular weight of 8kDa on an implant has not been previously reported. It is therefore surprising and unexpected that LMW-DexS stabilizes and increases the duration of bioactivity of immobilized fibrinolytic enzymes and/or releasable antimicrobial agent(s) on the surface of a medical device that interfaces with blood. As described herein LMW-DexS having a molecular weight of about 8,000 Da is part of the invention. However, it is disclosed LMW-DexS having a molecular weight of about 1,000, 2,000, 4,000, 10,000, and 20,000 Da. Advantages of various embodiments of the present invention include: 1) improvements in the biodurability of covalently immobilized fibrinolytic enzymes; 2) optimization of LMW-DexS/fibrinolytic enzyme compositions; 3) stabilization of immobilized fibrinolytic enzyme structure; 4) compatibility of the stabilized and immobilized fibrinolytic enzymes with antimicrobial agents such as chlorhexidine (CHX), gentian violet (GV), and/or brilliant green.

[0012] In addition, it is within the purview of this and other embodiments of the invention that other suitable agents may be incorporated into the bulk material. Examples of suitable agents includes other antimicrobial agents, antibiotics, antiseptics, chemotherapeutics, antimicrobial peptides, mimetics, antithrombogenic, fibrinolytic, anticoagulants, anti-inflammatory, anti-pain, antinausea, vasodilators, antiproliferatives, antifibrotics, growth factors, cytokines, antibodies, peptide and peptide mimetics, nucleic acids, and/or the like.

[0013] Medical devices suitable for use with various embodiments of the invention may include catheters, tubes, sutures, non-wovens, meshes, drains, shunts, stents, foams etc. Other devices suitable for use with embodiments of the invention include those that may interface with blood, blood products, and/or fibrinogenic fluids, tissues, and/or products. In various embodiments, the fibrinolytic enzyme and/or dextran sulfate may be incorporated in or on all or part of the medical device. In a particular example, the fibrinolytic enzyme and 8 kDa LMW-DexS may be applied to the tip area of a vascular catheter. In this manner, the amount of the relatively expensive enzyme may be reduced without adversely effecting the efficacy of the vascular catheter. Benefits of one or more embodiments of this invention include the stabilization and increase in the duration ofbioactivity of immobilized fibrinolytic enzymes and/or releasable agent(s) on the surface of a medical device that interfaces with blood.

[0014] Forms of chlorhexidine suitable for use with embodiments of the invention include chlorhexidine base, pharmaceutically acceptable chlorhexidine salts such as, for example, diacetate, laurate (dodecanoate), palmitate (hexadecanoate), myristate (tetradecanoate), stearate (octadecanoate) and/or the like. In addition, while particular examples are made of chlorhexidine base, chlorhexidine diacetate, and chlorhexidine dodecanoate, embodiments of the invention are not limited to any one form. Instead, as used herein, the term, 'chlorhexidine' refers to any one or a mixture of chlorhexidine base, pharmaceutically acceptable chlorhexidine salts such as, for example, diacetate, dodecanoate, palmitate, myristate, stearate and/or the like. For example, other suitable chlorhexidine salts are to be found in U.S. Patent 6,706,024, entitled Triclosan-Containing Medical Devices, issued on March 16, 2004, the disclosure of which is hereby incorporated in its entirety. In general, suitable concentrations of chlorhexidine include a range from about 0.1 % weight

to weight (wt/wt) to about 30% wt/wt. More particularly, a suitable chlorhexidine range includes from about 3% wt/wt to about 20% wt/wt.

[0015] Suitable base materials generally include elastomers and/orpolymer materials. Specific examples of suitable base materials include polyurethanes, polyvinylchlorides, thermoplastics such as, for example, fluoropolymers, vinyl polymers, polyolephins, copolymers, and/or the like.

[0016] In the following experiments, a duration of fibrinolytic activity is determined for urokinase-type plasminogen activator (uPA) immobilized on polyurethane catheter segments is determined. In addition, the unexpected improvements in the duration of fibrinolytic activity is determined for dextran sulfate stabilized uPA immobilized on polyurethane catheter segments is determined. We further show the unexpected improvements in the duration of fibrinolytic activity and antimicrobial activity when low molecular weight dextran sulfate is combined with uPA and an antimicrobial agent such as chlorhexidine and/or gentian violet.

METHODS

EXPERIMENT 1: Determining a duration of fibrinolytic activity for urokinase-type plasminogen activator (uPA) immobilized on polyurethane catheter segments

[0017] To immobilize the urokinase-type plasminogen activator (uPA), polyurethane catheter segments were treated with 1% Butadiene Maleic Anhydride (BMA1:1 ratio) polymer (25% solution in acetone, Polysciences, Warrington PA) and 1% Polyethylene glycol 400 (PEG 400, Hampton Research, Aliso Viejo CA) in acetone followed by curing at 90-100 C for 3 hours under vacuum. Subsequently, the cured segments were incubated for 18 hours in sodium acetate buffer containing uPA at 30 units/ μl. Thereafter, the segments were rinsed with phosphate buffered saline twice and finally with deionized water.

[0018] To test the durability of the fibrinolytic treatment, the catheters segments were incubated at 37°C in citrated human plasma for different time periods. The citrated human plasma was replaced with fresh sample after every 7 days. As shown in Figure 1, the catheter segments lost most of the fibrinolytic activity within 28 days.

EXPERIMENT 2: Determining *in vivo* performance of the fibrinolytic catheters implanted in the superior vena cava of rabbits

[0019] To assess *in vivo* performance of the fibrinolytic catheters, single lumen size 6 French (Fr) catheters were implanted in the superior vena cava of rabbits for 14 days. Urokinase activity was measured on the surface of explanted catheters via a chromogenic assay as is well known to those skilled in the art. As shown in Figure 2, the catheters retained about 20% of the original activity at day 14. The *in vivo* results correspond well with the amount of activity lost *in vitro* after fourteen days of incubation in human plasma (Figure 1).

EXPERIMENT 3: Evaluating compounds for improved performance of the fibrinolytic catheters

[0020] Various compounds were evaluated for improved duration of fibrinolytic activity. These compounds included: 1) low molecular weight dextran sulfate (LMW-DexS) with molecular weight of 8 kilo dalton (8 kDa); 2) human serum albumin (HSA); and 3) arginine. In this test, either 1 milligram/milliliter (mg/mL) of HSA or 1% weight/volume (w/v) of LMW-DexS (8 kDa) was added to a solution of sodium acetate buffer containing uPA at 30 units/μl. Either of the above solution was used to incubate BMA/PEG treated catheter segments. In addition, segments treated with BMA/PEG and uPA were rinsed in saline and, subsequently dipped in an aqueous solution containing 0.01% arginine as described in United States Patent No.: 4,764,466, entitled Method for stabilizing an immobilized fibrinolytic enzyme, issued on August 16, 1988.

[0021] As shown in Figure 3, LMW-DexS (8 kDa) treated segments retained the highest enzyme activity after 28 days of incubation in water at 37°C compared to the other treatments.

EXPERIMENT 4: Evaluating compounds for improved performance of the fibrinolytic catheters containing an antimicrobial agent

[0022] As disclosed in U.S. Patent Nos. 5,688,516, 6,273,875, and 6,528,107, medical devices may be treated with antimicrobial agents together with anticoagulants to provide dual benefits. However, to date, no known research has been conducted to examine the use of LMW-DexS (8 kDa) to improve the bio-durability of implantable medical devices with antimicrobial agents together with anticoagulants. To test if LMW-DexS (8 kDa) has similar stabilizing effect on immobilized urokinase in presence of an antimicrobial agent, polyurethane catheters were dip coated in a polymer solution containing either chlorhexidine diacetate (CHA) or chlorhexidine dodecanoate (CHDD) and dried overnight at room temperature. The CHA or CHDD coated catheters were subsequently dipped in BMA/PEG solution for 30 seconds and then cured for 3 hours at 90-100°C under vacuum. Thereafter the cured segments were incubated in urokinase solution containing HSA, LMW-DexS (8 kDa) or arginine as in the earlier experiment.

[0023] As shown in Figure 4, of the three agents tested, LMW-DexS (8 kDa) treated segments maintained the highest urokinase activity after 28 days of incubation in water.

EXPERIMENT 5: Evaluating the use of maleic anhydride to attach urokinase to the catheters

[0024] Thin films rich in anhydride groups can be created by pulsed plasma polymerization of maleic anhydride. (See J Hu et al (2003) Functionalization ofpoly(ethylene terephthalate) film by pulsed plasma deposition of maleic anhydride. Advanced Functional Materials 13: 692- 697.) The plasma of maleic anhydride was utilized as a method to introduce anhydride groups for enzyme attachment on to the CHA/polyurethane surface. This method of enzyme attachment is generally known to those skilled in the art and thus, in the interest of brevity, is only briefly described herein. Polyurethane catheters were dip coated in a polymer solution containing CHA, dried overnight at room temperature, treated with maleic anhydride (Sigma-Aldrich Corp. St. Louis MO) plasma generated under Argon atmosphere in a plasma chamber (Diener Electronics, Reading PA). The plasma deposition reactions were carried out at a pressure of 15 Pascal (Pa), 90 Watts (W) peak power for 5 minutes or for ten cycles of 12 seconds each. Subsequently, catheter segments were incubated for 18 hours in sodium acetate buffer, pH 5 containing 30 units/μl uPA with or without 1% LMW-DexS (8 kDa). Catheter segments were rinsed in phosphate buffered saline (PBS) twice and then in deionized water before incubation in citrated human plasma for durability testing. After 28 days of incubation in human plasma, fibrinolytic activity of catheter segments was assessed by a clot lysis method. The clots were made as follows: 9% fibrinogen and 1.7 units/mL of plasminogen were incubated at 37°C for 5 minutes in 197 millimolar (mM) borate/borax buffer, pH 7.5 containing 0.9% (w/v) sodium chloride and 0.5% (w/v) gelatin. Subsequently, 100units/mL of thrombin was added to initiate the fibrin polymerization and clot formation.

[0025] To conduct the clot lysis assay, a 0.5 cm long catheter segment was placed on a clot for 6 hours at 37°C and the volume of lysed clot was recorded. This procedure was performed for each sample shown in Figure 5. As shown in Figure 5, approximately an eight fold higher clot lysis was achieved when 1% LMW-DexS (8 kDa) was included as enzyme stabilizer compared to high molecular weight dextran sulfate or absence of dextran sulfate after 9 days of soaking in human plasma.

EXPERIMENT 6: Evaluating the duration of fibrinolytic activity for low molecular weight dextran sulfate stabilized catheter segments containing chlorhexidine diacetate

[0026] To evaluate the durability of the 1% LMW-DexS (8 kDa) treated catheters segments, the catheter segments were incubated at 37°C in citrated human plasma for different time periods. The citrated human plasma was replaced with fresh sample after every 7 days. As shown in Figure 6, the 1% LMW-DexS (8 kDa) treated catheters segments retained approximately 80% of the fibrinolytic activity after 28 days. That is, the 1% LMW-DexS (8 kDa) stabilized and immobilized urokinase enzyme remained active for 28 days in human plasma substantially without any loss in the activity compared to the control. These results were particularly unexpected in light of the approximately 90+% loss in activity shown in Figures 1 and 6 for un-stabilized urokinase.

EXPERIMENT 7: Evaluating antimicrobial activity for low molecular weight dextran sulfate stabilized catheter segments containing antimicrobial agents

[0027] In addition to fibrinolytic activity, antimicrobial activity of the 1% LMW-DexS (8 kDa) treated catheters was evaluated. In this test, adherence of gram positive bacteria *Staphylococcus aureus* ATCC 33591, on the catheter segments having immobilized urokinase stabilized with 1 % LMW-DexS (8 kDa), along with the antimicrobial agents such as gentian violet (0.6%) and chlorhexidine dodecanoate was compared to similarly treated catheter segments but without the LMW-DexS (8 kDa) stabilized enzyme. A five log reduction in adherence of the bacteria was observed on both catheter types, i.e. either with or without LMW-DexS (8 kDa). Thus, the antimicrobial activity of gentian violet and chlorhexidine dodecanoate against the tested organisms was not compromised due to the attachment of LMW-DexS (8 kDa) stabilized urokinase. Additional disclosed embodiments are summarized in the following statements:

A. A medical device comprising:

> a base material including an immobilized fibrinolytic enzyme and dextran sulfate, the dextran sulfate having a molecular weight that is less than 40 kilo dalton (kDa).

B. The medical device according to Statement A, wherein the fibrinolytic enzyme is covalently bound to the base material.

C. The medical device according to Statement A, wherein the fibrinolytic enzyme includes a urokinase-type plasminogen activator (uPA).

D. The medical device according to Statement A, further comprising:

> a combination of two or more antimicrobial agents impregnating the base material.

E. The medical device according to Statement D, wherein the combination of two or more antimicrobial agents includes chlorhexidine base and a pharmaceutically acceptable salt thereof.

F. The medical device according to Statement A, further comprising:

an antimicrobial agent impregnating the base material.

G. The medical device according to Statement F, wherein the antimicrobial agent includes chlorhexidine base or a pharmaceutically acceptable salt thereof.

H. The medical device according to Statement F, wherein the antimicrobial agent includes chlorhexidine diacetate.

I. The medical device according to Statement F, wherein the antimicrobial agent includes chlorhexidine dodecanoate.

J. The medical device according to Statement F, wherein the antimicrobial agent includes gentian violet.

K. The medical device according to Statement A, wherein the dextran sulfate has a molecular weight of about 8 kDa.

L. The medical device according to Statement A, further comprising:

a tubular structure including the base material.

M. The medical device according to Statement A, wherein the base material is a polymer.

N. The medical device according to Statement M, wherein the base material is a polyurethane.

O. A medical device comprising:

a polyurethane base material;
an amount of a fibrinolytic enzyme sufficient to reduce the formation of blood clots in a patient, the fibrinolytic enzyme being immobilized in the base material;
a low molecular weight dextran sulfate to stabilize the fibrinolytic enzyme, the low molecular weight dextran sulfate having a molecular weight of less than 40 kilo dalton; and
a chlorhexidine base and/or a pharmaceutically acceptable salt thereofbeing disposed in the polyurethane base material in an amount sufficient to reduce microbial growth.

P. A medical device comprising:

a polyurethane base material;
an amount of a urokinase-type plasminogen activator (uPA) sufficient to reduce the formation of blood clots in a patient, the uPA being covalently immobilized in the base material;
a low molecular weight dextran sulfate to stabilize the fibrinolytic enzyme, the low molecular weight dextran sulfate having a molecular weight of less than 40 kilo dalton (kDa); and
a chlorhexidine base and/or a pharmaceutically acceptable salt thereofbeing disposed in the polyurethane base material in an amount sufficient to reduce microbial growth.

Q. A method of fabricating a medical device, the method comprising:

forming the medical device from at least a base material;
immobilizing a fibrinolytic enzyme on the base material; and
stabilizing the fibrinolytic enzyme with a dextran sulfate having a molecular weight of less than 40 kilo dalton (kDa).

R. The method according to Statement Q, further comprising:

incubating the medical device in a fibrinolytic solution of the fibrinolytic enzyme and dextran sulfate.

S. The method according to Statement P, further comprising:

preparing the fibrinolytic solution by dissolving about 30 units per micro liter of the fibrinolytic enzyme in a sodium acetate buffer, the fibrinolytic enzyme including urokinase-type plasminogen activator (uPA).

T. The method according to Statement Q, further comprising:

adding about 1% of the dextran sulfate to the fibrinolytic solution.

U. The method according to Statement Q, further comprising:

adding about 1 % of the dextran sulfate having a molecular weight of about 8 kDa to the fibrinolytic solution.

V. The method according to Statement Q, further comprising:

treating the base material to generate binding sites for covalently binding the fibrinolytic enzyme to the base material.

W. The method according to Statement Q, further comprising:

impregnating the base material with a combination of two or more antimicrobial agents.

X. The method according to Statement W, further comprising:

impregnating the base material with the combination of chlorhexidine base and a pharmaceutically acceptable salt thereof.

Y. The method according to Statement Q, further comprising:

impregnating the base material with an antimicrobial agent.

Z. The method according to Statement Y, further comprising:

impregnating the base material with a chlorhexidine base or a pharmaceutically acceptable salt thereof.

AA. The method according to Statement Y, further comprising:

impregnating the base material with chlorhexidine diacetate.

AB. The method according to Statement Y, further comprising:

impregnating the base material with chlorhexidine dodecanoate.

AC. The method according to Statement Y, further comprising:

impregnating the base material with gentian violet.

AD. The method according to Statement Y, further comprising:

impregnating the base material with brilliant green.

AE. The method according to Statement Q, further comprising:

forming a tubular structure from the base material.

AF. The method according to Statement Q, further comprising:

wherein the base material includes a polymer.

AG. The method according to Statement AF, further comprising:

wherein the polymer includes polyurethane.

AH. The method according to Statement Q, further comprising:

immobilizing the fibrinolytic enzyme on a tip of the medical device.

Claims

1. A medical device comprising:

a base material including an immobilized fibrinolytic enzyme covalently bound to the base material and dextran sulfate, the dextran sulfate having a molecular weight that is 8 kilo dalton (kDa) to stabilize the fibrinolytic enzyme when in contact with blood of the patient, wherein the fibrinolytic enzyme includes a urokinase-type plasminogen activator (uPA).

2. The medical device according to claim 1, further comprising:

a combination of two or more antimicrobial agents impregnating the base material.

3. The medical device according to claim 2, wherein the combination of two or more antimicrobial agents includes chlorhexidine base and a pharmaceutically acceptable salt thereof.

4. The medical device according to claim 3, wherein the combination of two or more antimicrobial agents includes chlorhexidine diacetate, chlorhexidine dodecanoate, gentian violet, or brilliant green.

5. The medical device according to claim 1, further comprising:

a tubular structure including the base material.

6. The medical device according to claim 1, wherein the base material is a polymer.

7. The medical device according to claim 6, wherein the base material is a polyurethane.

8. The medical device according to claim 1, wherein the base material is a polyurethane;
the fibrinolytic enzyme being an amount sufficient to reduce the formation of blood clots in a patient;
the dextran sulfate stabilizing the fibrinolytic enzyme, and

a chlorhexidine base and/or a pharmaceutically acceptable salt thereof being disposed in the polyurethane base material in an amount sufficient to reduce microbial growth.

9. The medical device according to claim 8, wherein the base material is a polyurethane;
an amount of a urokinase-type plasminogen activator (uPA) sufficient to reduce the formation of blood clots in a patient, the uPA being covalently immobilized in the base material;
the dextran sulfate stabilizing the fibrinolytic enzyme; and
a chlorhexidine base and/or a pharmaceutically acceptable salt thereof being disposed in the polyurethane base material in an amount sufficient to reduce microbial growth.

10. A method of fabricating a medical device according to any one of the claims 1-10 including:

forming the medical device from at least the base material;
immobilizing the fibrinolytic enzyme by covalently binding the fibrinolytic enzyme on the base material; and
stabilizing the fibrinolytic enzyme with dextran sulfate having a molecular weight of 8kilo dalton (kDa), wherein
the fibrinolytic enzyme includes a urokinase-type plasminogen activator (uPA).

11. The method according to claim 10, further comprising;
incubating the medical device in a fibrinolytic solution of the fibrinolytic enzyme and dextran sulphate.

12. The method according to claim 11, further comprising;
preparing the fibrinolytic solution by dissolving about 30 units per micro liter of the fibrinolytic enzyme in a sodium acetate buffer, the fibrinolytic enzyme including urokinase-type plasminogen activator (uPA).

13. The method according to claim 12, further comprising:

adding about 1% of the dextran sulfate to the fibrinolytic solution.

14. The method according to claim 14, further comprising:

adding about 1% of the dextran sulfate having a molecular weight of about 8 kDa to the fibrinolytic solution.

15. The method according to claim 13, further comprising:

treating the base material to generate binding sites for covalently binding the fibrinolytic enzyme to the base material.

16. The method according to claim 13, further comprising:

immobilizing the fibrinolytic enzyme on a tip of the medical device.

Patentansprüche

1. Medizinprodukt, umfassend:

ein Basismaterial, das ein immobilisiertes fibrinolytisches Enzym einschließt,
kovalent gebunden an das Basismaterial und Dextransulfat, wobei das Dextransulfat ein Molekulargewicht aufweist, das 8 kiloDalton (kDa) ist, um das fibrinolytische Enzym zu stabilisieren, wenn es in Kontakt mit Blut des Patienten ist, wobei
das fibrinolytische Enzym einen Plasminogenaktivator vom Urokinasetyp (uPA) einschließt.

2. Medizinprodukt nach Anspruch 1, ferner umfassend:

eine Kombination von zwei oder mehr antimikrobiellen Mitteln, die das Basismaterial imprägnieren.

3. Medizinprodukt nach Anspruch 2, wobei die Kombination von zwei oder mehr antimikrobiellen Mitteln Chlorhexidin-Base und ein pharmazeutisch annehmbares Salz davon einschließt.

4. Medizinprodukt nach Anspruch 3, wobei die Kombination von zwei oder mehr antimikrobiellen Mitteln Chlorhexidindiacetat, Chlorhexidindodecanoat, Gentianaviolett oder Brilliantgrün einschließt.

5. Medizinprodukt nach Anspruch 1, ferner umfassend eine rohrförmige Struktur, die das Basismaterial einschließt.

6. Medizinprodukt nach Anspruch 1, wobei das Basismaterial ein Polymer ist.

7. Medizinprodukt nach Anspruch 6, wobei das Basismaterial ein Polyurethan ist.

8. Medizinprodukt nach Anspruch 1, wobei das Basismaterial ein Polyurethan ist;
das fibrinolytische Enzym eine ausreichende Menge

ist, um die Bildung von Blutgerinnseln in einem Patienten zu reduzieren;
das Dextransulfat das fibrinolytische Enzym stabilisiert und
eine Chlorhexidin-Base und/oder ein pharmazeutisch annehmbares Salz davon in einer ausreichenden Menge in dem Polyurethanbasismaterial angeordnet ist, um mikrobielles Wachstum zu reduzieren.

9. Medizinprodukt nach Anspruch 8, wobei das Basismaterial ein Polyurethan ist;
eine Menge eines Plasminogenaktivators vom Urokinasetyp (uPA), die ausreicht, um die Bildung von Blutgerinnseln in einem Patienten zu reduzieren, wobei der uPA in dem Basismaterial kovalent immobilisiert ist;
das Dextransulfat das fibrinolytische Enzym stabilisiert und
eine Chlorhexidin-Base und/oder ein pharmazeutisch annehmbares Salz davon in einer ausreichenden Menge in dem Polyurethanbasismaterial angeordnet ist, um mikrobielles Wachstum zu reduzieren.

10. Verfahren zum Fertigen eines Medizinprodukts nach einem der Ansprüche 1-10, welches einschließt:

Bilden des Medizinprodukts aus mindestens dem Basismaterial;
Immobilisieren des fibrinolytischen Enzyms durch kovalentes Binden des fibrinolytischen Enzyms an das Basismaterial und
Stabilisieren des fibrinolytischen Enzyms mit Dextransulfat mit einem Molekulargewicht von 8 kiloDalton (kDa), wobei
das fibrinolytische Enzym einen Plasminogenaktivator vom Urokinasetyp (uPA) einschließt.

11. Verfahren nach Anspruch 10, ferner umfassend:

Inkubieren des Medizinprodukts in einer fibrinolytischen Lösung von dem fibrinolytischen Enzym und Dextransulfat.

12. Verfahren nach Anspruch 11, ferner umfassend:

Zubereiten der fibrinolytischen Lösung durch Auflösen von etwa 30 Einheiten des fibrinolytischen Enzyms pro Mikroliter in einem Natriumacetatpuffer, wobei das fibrinolytische Enzym Plasminogenaktivator vom Urokinasetyp (uPA) einschließt.

13. Verfahren nach Anspruch 12, ferner umfassend:

Zufügen von etwa 1 % des Dextransulfats zu der fibrinolytischen Lösung.

14. Verfahren nach Anspruch 14, ferner umfassend:

Zufügen von etwa 1 % des Dextransulfats mit einem Molekulargewicht von etwa 8 kDa zu der fibrinolytischen Lösung.

15. Verfahren nach Anspruch 13, ferner umfassend:

Behandeln des Basismaterials, um Bindungsstellen zum kovalenten Binden des fibrinolytischen Enzyms an das Basismaterial zu generieren.

16. Verfahren nach Anspruch 13, ferner umfassend:

Immobilisieren des fibrinolytischen Enzyms auf einer Spitze des Medizinprodukts.

Revendications

1. Dispositif médical comprenant :

un matériau de base incluant une enzyme fibrinolytique immobilisée liée de manière covalente au matériau de base et du sulfate de dextran, le sulfate de dextran ayant un poids moléculaire qui est de 8 kilo dalton (kDa) pour stabiliser l'enzyme fibrinolytique lorsqu'elle est en contact avec du sang du patient, dans lequel
l'enzyme fibrinolytique inclut un activateur du plasminogène de type urokinase (uPA).

2. Dispositif médical selon la revendication 1, comprenant en outre :

une combinaison de deux ou plus agents antimicrobiens imprégnant le matériau de base.

3. Dispositif médical selon la revendication 2, dans lequel la combinaison de deux ou plus agents antimicrobiens inclut une base de chlorhexidine et un sel pharmaceutiquement acceptable de celle-ci.

4. Dispositif médical selon la revendication 3, dans lequel la combinaison de deux ou plus agents antimicrobiens inclut le diacétate de chlorhexidine, le dodécanoate de chlorhexidine, le violet de gentiane, ou le vert brillant.

5. Dispositif médical selon la revendication 1, comprenant en outre :

une structure tubulaire incluant le matériau de base.

6. Dispositif médical selon la revendication 1, dans lequel le matériau de base est un polymère.

7. Dispositif médical selon la revendication 6, dans le-

quel le matériau de base est un polyuréthane.

8. Dispositif médical selon la revendication 1, dans lequel le matériau de base est un polyuréthane ;
l'enzyme fibrinolytique étant une quantité suffisante pour réduire la formation de caillots sanguins chez un patient ;
le sulfate de dextran stabilisant l'enzyme fibrinolytique, et
une base de chlorhexidine et/ou un sel pharmaceutiquement acceptable de celle-ci étant disposée dans la matériau de base en polyuréthane en une quantité suffisante pour réduire la croissance microbienne.

9. Dispositif médical selon la revendication 8, dans lequel le matériau de base est un polyuréthane ;
une quantité d'activateur du plasminogène de type urokinase (uPA) suffisante pour réduire la formation de caillots sanguins chez un patient, l'uPA étant immobilisé de manière covalente dans le matériau de base ;
le sulfate de dextran stabilisant l'enzyme fibrinolytique ; et
une base de chlorhexidine et/ou un sel pharmaceutiquement acceptable de celle-ci étant disposé dans le matériau de base en polyuréthane en une quantité suffisante pour réduire la croissance microbienne.

10. Procédé de fabrication d'un dispositif médical selon l'une quelconque des revendications 1 à 10 incluant :

former le dispositif médical à partir au moins du matériau de base ;
immobiliser l'enzyme fibrinolytique en liant de manière covalente l'enzyme fibrinolytique sur le matériau de base ; et
stabiliser l'enzyme fibrinolytique avec du sulfate de dextran ayant un poids moléculaire de 8 kilo dalton (kDa), dans lequel
l'enzyme fibrinolytique inclut un activateur du plasminogène de type urokinase (uPA).

11. Procédé selon la revendication 10, comprenant en outre :

incuber le dispositif médical dans une solution fibrinolytique de l'enzyme fibrinolytique et du sulfate de dextran.

12. Procédé selon la revendication 11, comprenant en outre :

préparer la solution fibrinolytique en dissolvant environ 30 unités par microlitre de l'enzyme fibrinolytique dans un tampon d'acétate de sodium, l'enzyme fibrinolytique incluant un activateur du plasminogène de type urokinase (uPA).

13. Procédé selon la revendication 12, comprenant en outre :

ajouter environ 1% du sulfate de dextran à la solution fibrinolytique.

14. Procédé selon la revendication 14, comprenant en outre :

ajouter environ 1% du sulfate de dextran ayant un poids moléculaire d'environ 8 kDa à la solution fibrinolytique.

15. Procédé selon la revendication 13, comprenant en outre :

traiter le matériau de base pour générer des sites de liaison pour lier de manière covalente l'enzyme fibrinolytique au matériau de base.

16. Procédé selon la revendication 13, comprenant en outre :

immobiliser l'enzyme fibrinolytique sur une pointe du dispositif médical.

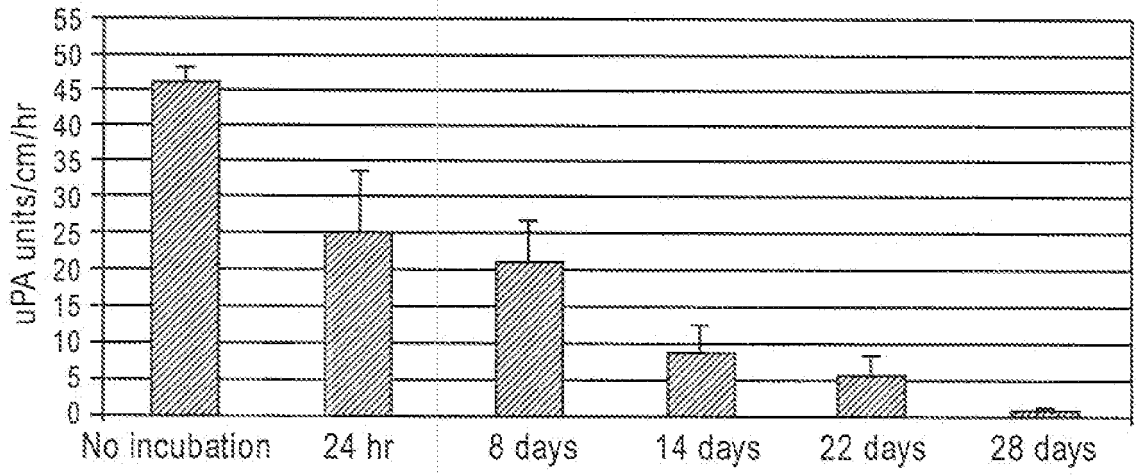

uPA units/cm/hr
55
50
45
40
35
30
25
20
15
10
5
0
No incubation
24 hr
8 days
14 days
22 days
28 days

FIG. 1

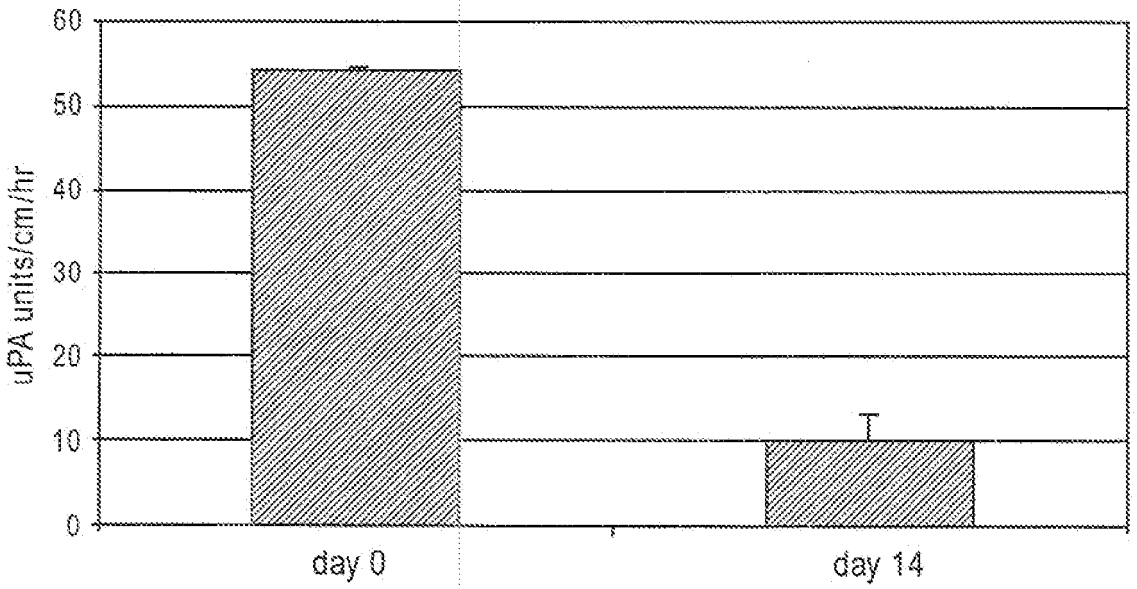

uPA units/cm/hr
60
50
40
30
20
10
0
day 0
day 14

FIG. 2

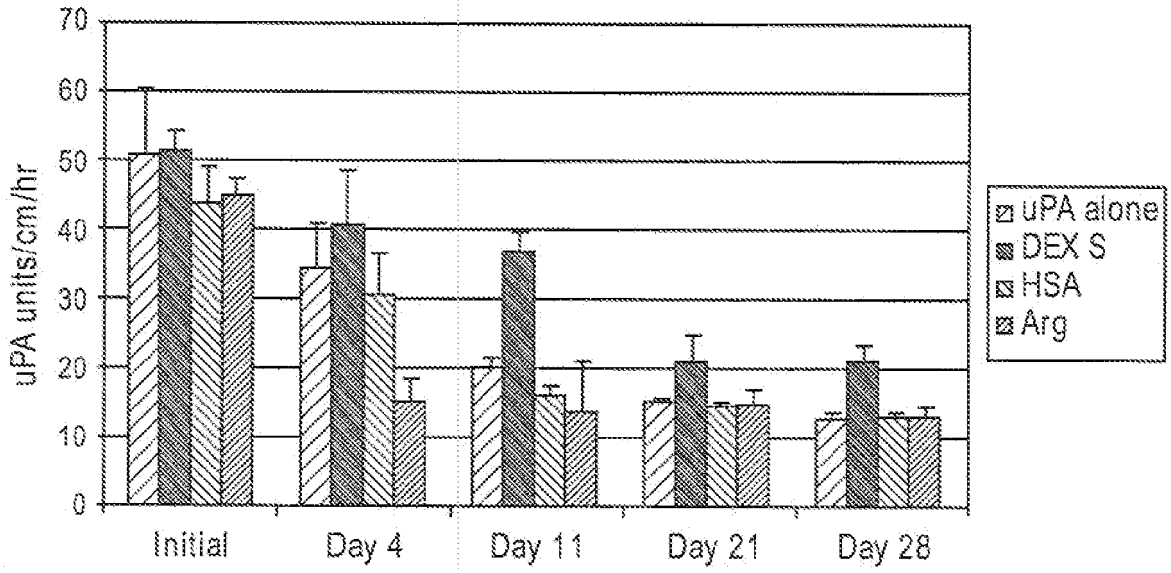

70
60
50
40
30
20
10
0
uPA units/cm/hr
Initial
Day 4
Day 11
Day 21
Day 28
uPA alone
DEX S
HSA
Arg

FIG. 3

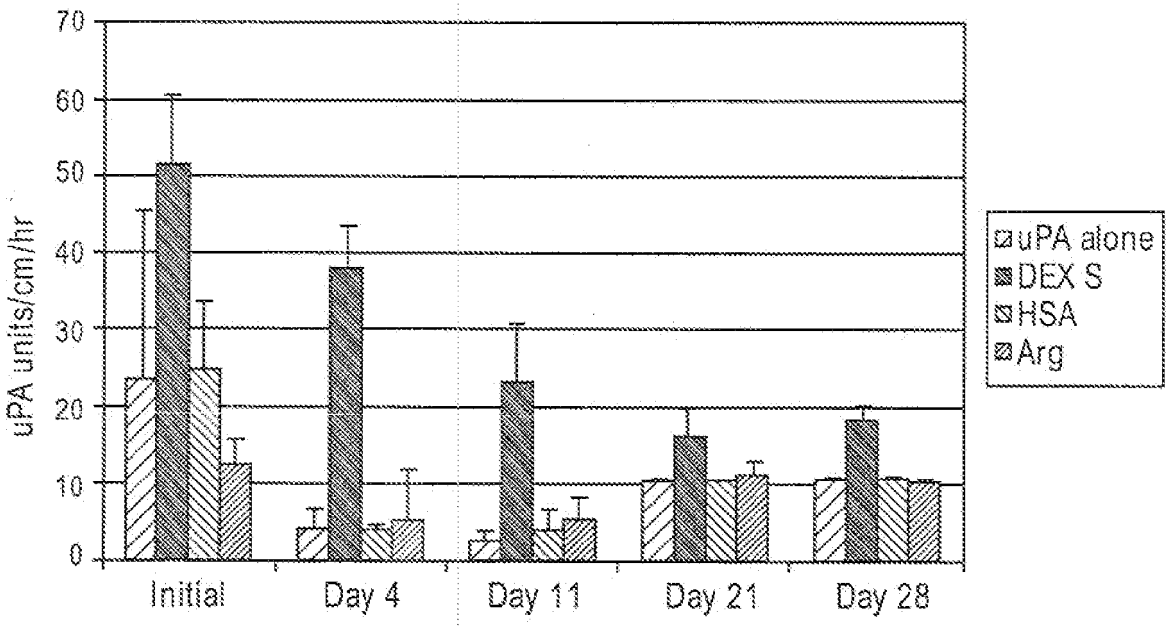

70
60
50
40
30
20
10
0
uPA units/cm/hr
Initial
Day 4
Day 11
Day 21
Day 28
uPA alone
DEX S
HSA
Arg

FIG. 4

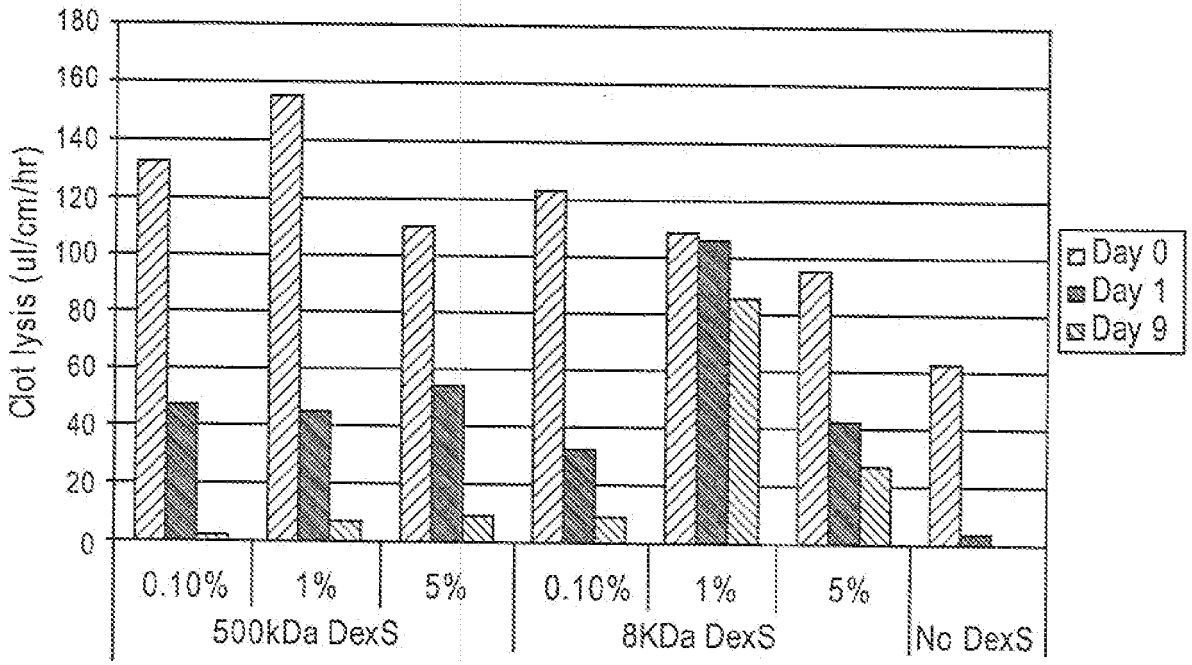

Clot lysis (ul/cm/hr)
180
160
140
120
100
80
60
40
20
0
0.10%
1%
5%
0.10%
1%
5%
500kDa DexS
8KDa DexS
No DexS
Day 0
Day 1
Day 9

FIG. 5

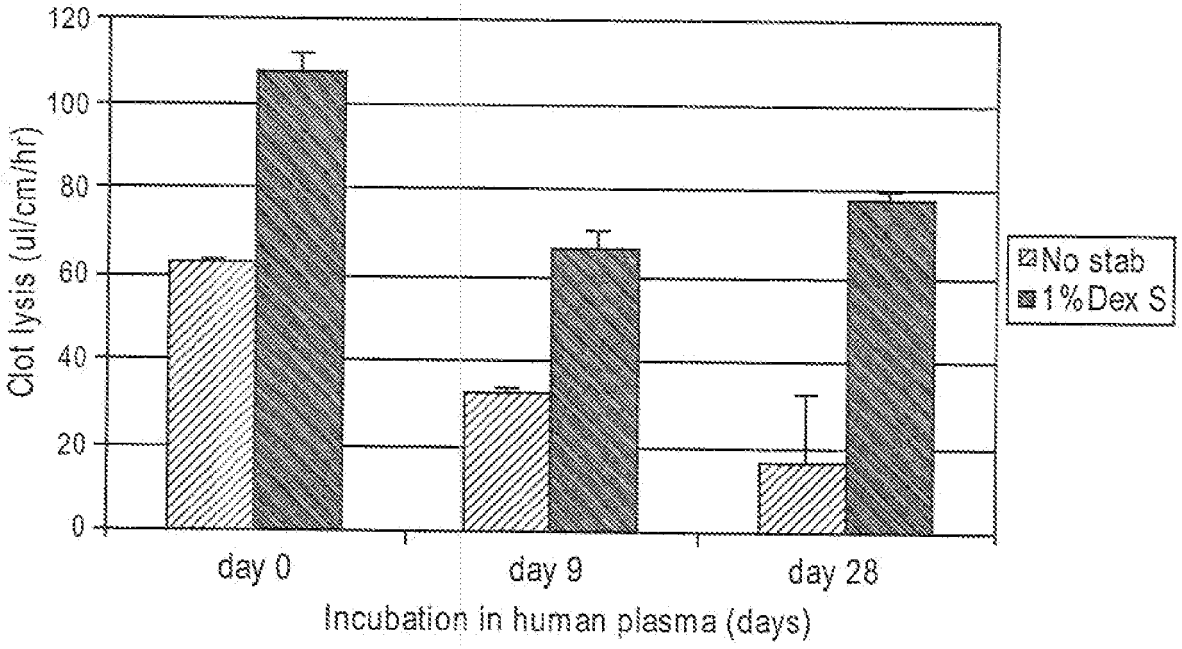

Clot lysis (ul/cm/hr)
120
100
80
60
40
20
0
day 0
day 9
day 28
Incubation in human plasma (days)
No stab
1% Dex S

FIG. 6

REFERENCES CITED IN THE DESCRIPTION

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